Characterization of dorsal root ganglion neurons cultured on silicon micro-pillar substrates for high-resolution electrophysiological recordings

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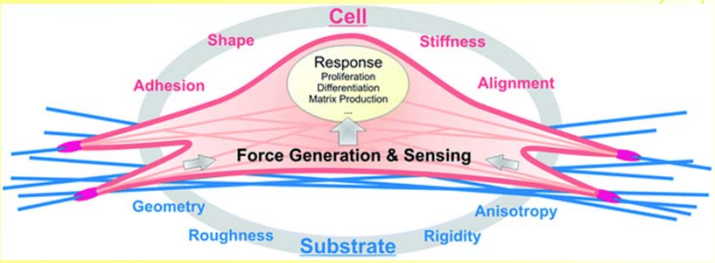
BACKGROUND AND AIMS

Generally: Cells effectively sense the chemical and physical cues in their microenvironment and respond accordingly by altering their properties



***In vivo:* Extracellular matrix**

- cells are exposed to a three dimensional ECM with a variety of geometrically-defined micro- and nano-scale components
  → physical cues or topographies

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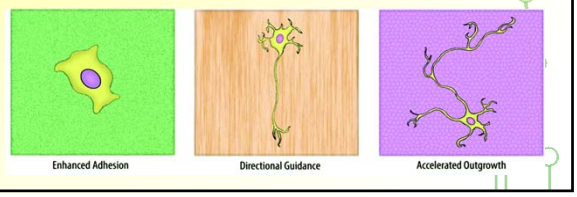
***In vitro:* Substrate topography**

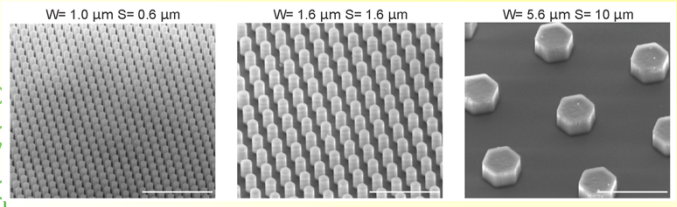
- affects cell morphology, adhesion, migration, proliferation and differentiation

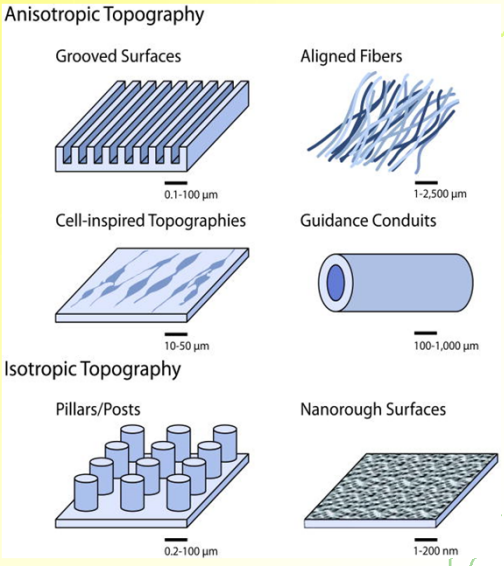


BACKGROUND AND AIMS

- variety of different micro- and nano- patterned substrates were used in cellular behavior studies
- the same micro-pattern could have different effects in different types of neurons

→ what about effect on different developmental stages?
→ what about effect on different neuronal subtypes?





hexagonally-shaped micro-pillar substrates

AIMS:

To examine the effect of micro-pillar substrate (MPS) topography on growth and morphology of dorsal root ganglia neurons (DRG)

i. To examine if differences between adult and neonatal DRG neuronal growth and morphology exist

ii. To examine if differences between main DRG neuronal subtypes exist

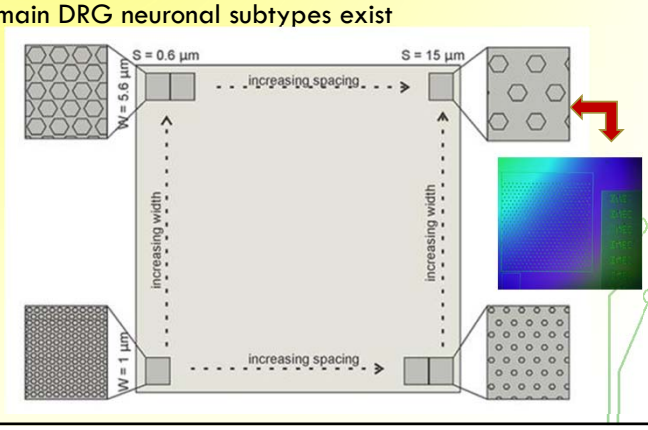
- MPS design – 150 areas

hexagonal micro-pillars:

equally high (3 µm)

different width (1-5.6 µm)

different spacing (0.6-15 µm)



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- MPS design – 150 areas

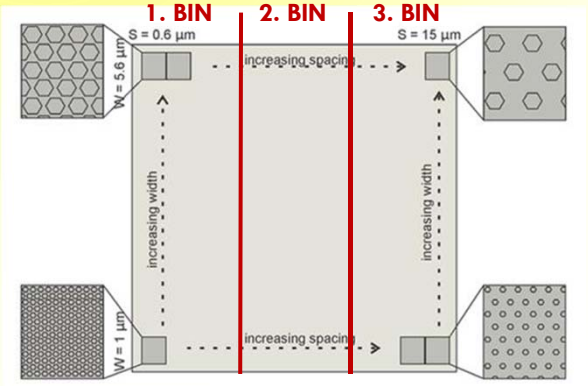
  hexagonal micro-pillars:

  equally high (3 μm)

  different width (1-5.6 μm)

  different spacing (0.6-15 μm)

  devided into 3 bins

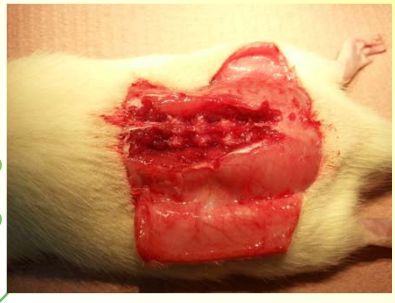


METHODS

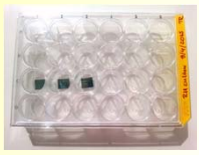
DRG harvesting

- From **adult** (170-220 g) Sprague-Dawley rats
- General anesthesia:

  2% isoflurane in oxygen
  (5% for inducing)



- From **neonatal** 5 to 7 day-old Sprague-Dawley rats
- Euthanized by decapitation under cold anesthesia



DRG neurons culturing

- Cultivation substrates: MPS and control glass coverslips
- Coating with poly-L-lysine
- Cell dissociation in trypsin/liberase/DNase solution
- Seeding density: 5000-15000 cells per MPS/coverslip
- Cultivation process:1, 3 and 7 day *in vitro* (DIV)

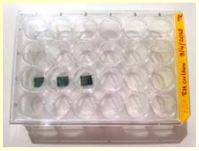
METHODS

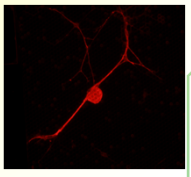
Immunocytochemistry

- Specific DRG neuronal subtypes were identified by simultaneous staining of NeuN and N52 or IB4 or CGRP

Table 1: Primary antibodies and conjugates used.

Primary antibodies and conjugates	Target	Manufacturer (cat. no.; lot. no.)	Dilution used
Rabbit polyclonal anti-neural nuclear antigen (NeuN)	All neurons	Sigma-Aldrich; SAB 4300883, lot 480132093	1:500
Mouse monoclonal anti-neurofilament 200 kDa, clone N52	Myelinated A-fiber DRG neurons	Milipore; MAB 5266, lot 2567008	1:300
Goat polyclonal anti-calcitonin gene-related peptide (CGRP)	Peptidergic A and C-fiber DRG neurons	Santa Cruz Biotechnology; Sc-8856, lot F1714	1:300
Isolectin B4 (IB4), FITC-conjugated	Nonpeptidergic C-fiber DRG neurons	Sigma-Aldrich; L 2895, lot 052M4086V	1:100

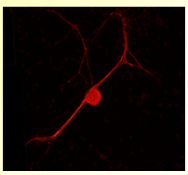




METHODS

Analysis of DRG neuronal distribution on MPS

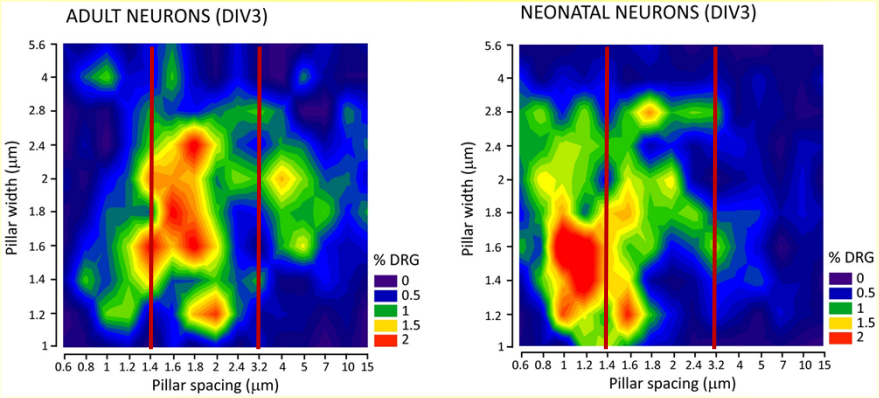
- Examine if there is any difference in neuronal growth depanding on pillar dimensions
- NeuN-positive cells were counted under fluorescent microscope
- Cell number in each MPS area was divided by total cell number to get cumulative percentages
- SigmaPlot software – graph as function of pillar width and spacing → surface plot



SIGMAPLOT
Exact Graphs for Exact Science

RESULTS

MPS topography affects DRG neuronal distribution

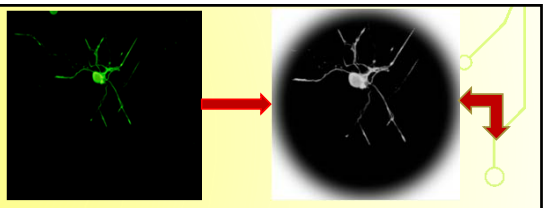


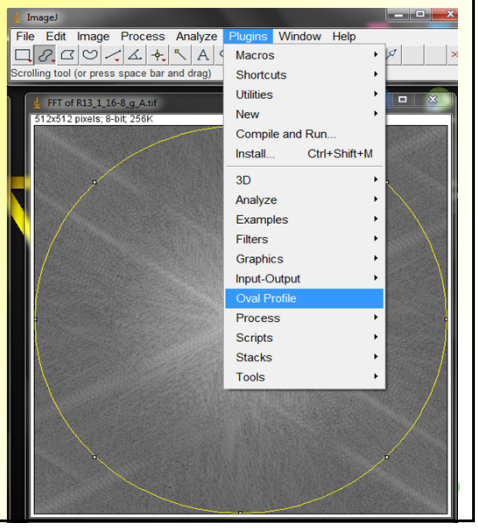
2. BIN **1. BIN**

- Geometrical features of MPS influence on DRG neuronal presence – distribution is not uniform!

METHODS

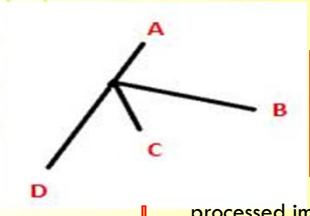
Quantification of neurite alignment on MPS and control glass surface





- NIH ImageJ software with „Oval profile“ plugin
- By performing Fast Fourier Transformation (FFT) – converts informaton present in an original data image from “real space” into mathematically defined “frequency” space
- Resulting FFT output image represents the pixel intensity that are distributed in pattern that reflects the degree of alignment present in original image

Original image



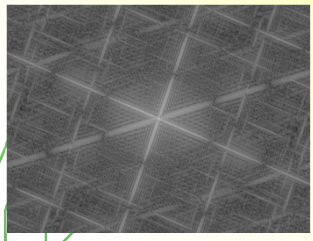
FFT_LINE TEST

PEAKS → ALIGNMENT

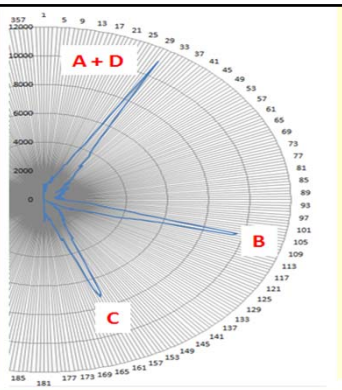
NO PEAKS → NO ALIGNMENT

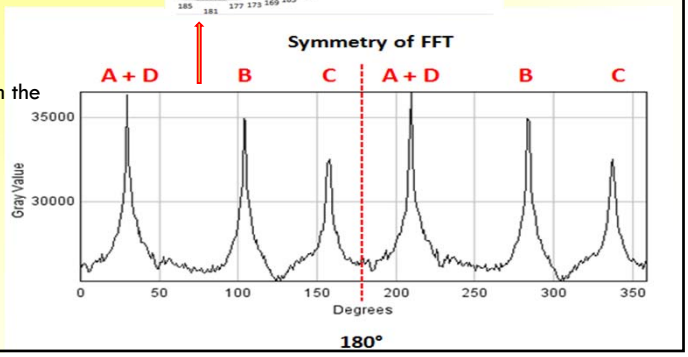
processed image is converted into frequency domain image by an FFT algorithm…

FFT image



…and then plotted as a spectrum by summing all values of each pixel from the center of the FFT image

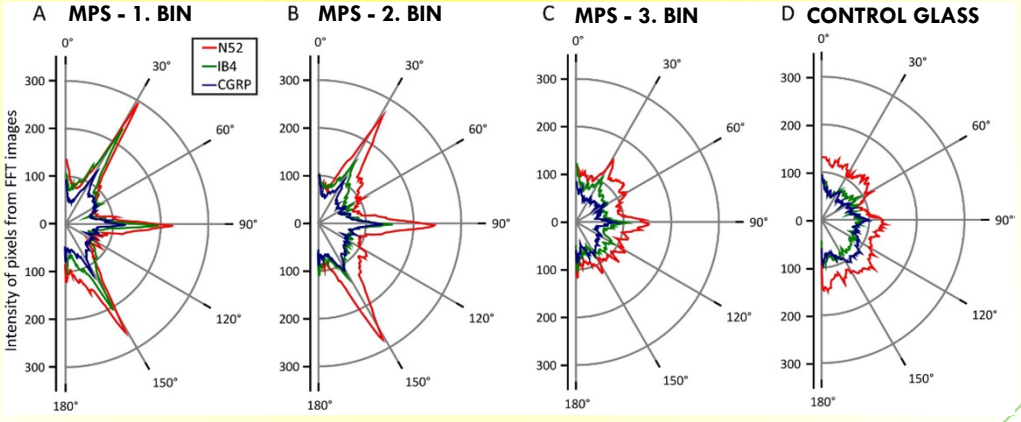




RESULTS

MPS spatial density affects neurite alignment of all DRG neuronal subtypes

ADULT

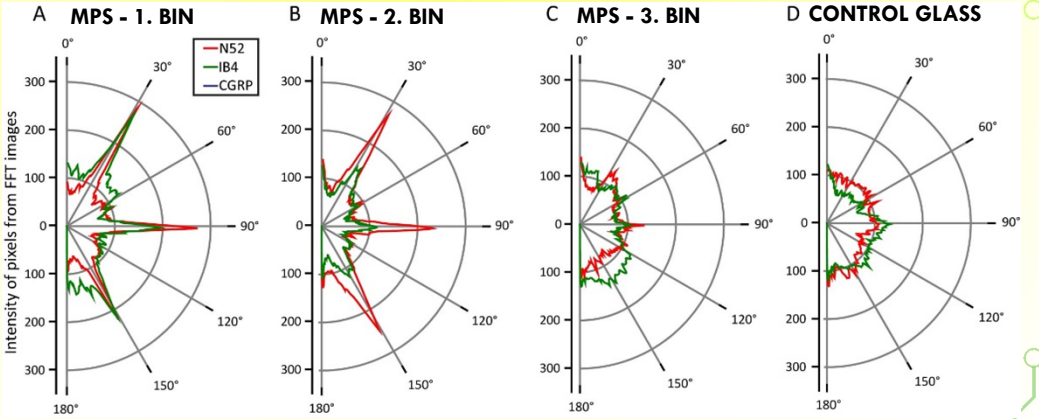


- MPS – 1. BIN and 2. BIN → strong influence on neurite guidance
- MPS – 3. BIN and control glass surface → no observable guidance

RESULTS

MPS spatial density affects DRG neurite alignment

NEONATAL



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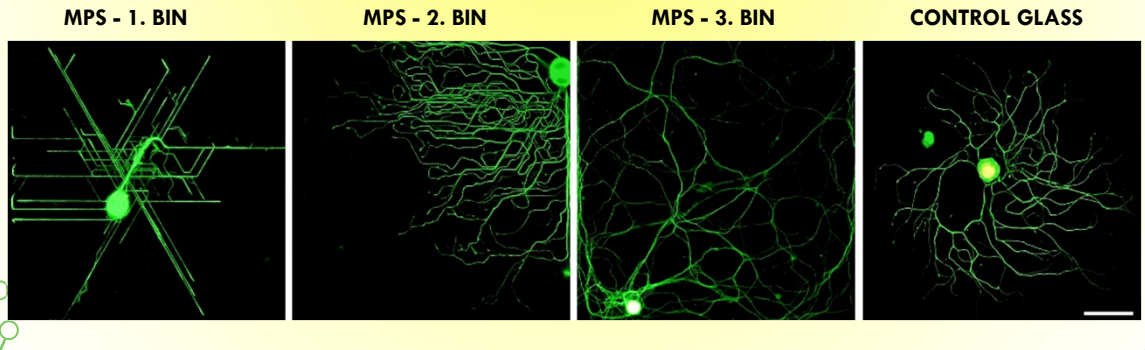
RESULTS

MPS spatial density affects DRG neurite alignment

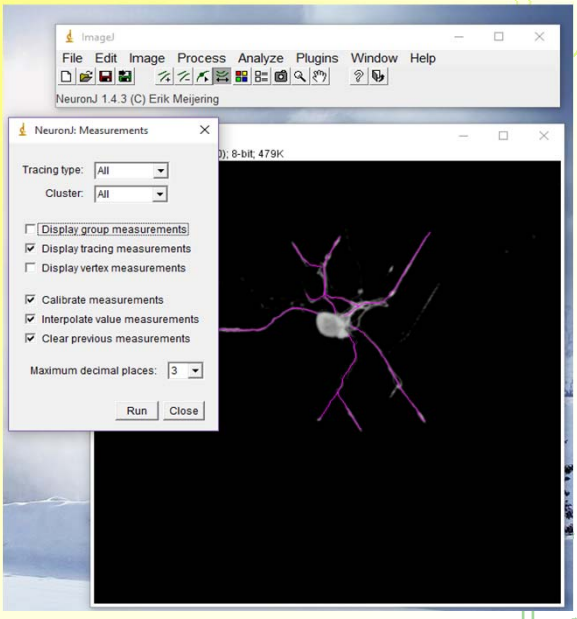


- From a visual examination – differences in neurite alignment are observed

METHODS

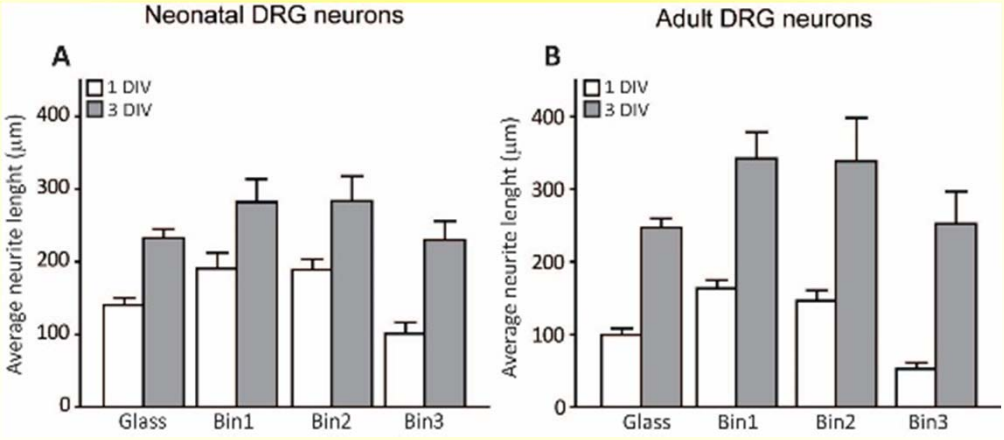
Measurement of neurite length on MPS and control glass surface

- NIH ImageJ software with „NeuronJ“ plugin
- Average neurite length per cell was obtained by manually tracing the length of all neurite outgrowths, divided by total number of neurites



RESULTS

MPS spatial density affects DRG neurite length

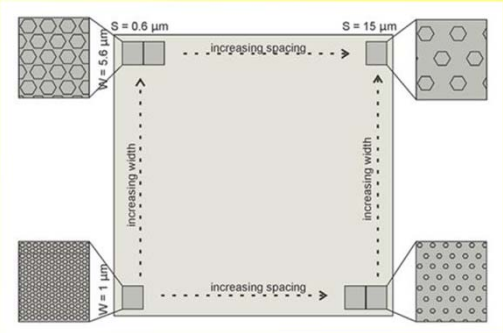


CONCLUSIONS

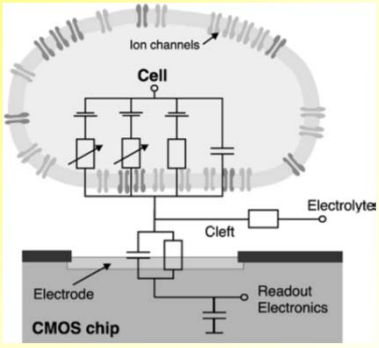
- Generally, micro-pillar substrate (MPS) topography affects growth and morphology of DRG neurons, in contrast to control glass coverslips
- Micro-pillars of particular size-range (0.6 – 1.4µm and 1.6 – 3.2µm) were optimal in promoting DRG neuronal presence, neurite growth and alignment
- There is no significante difference in
  - morphology of adult and neonatal DRG neurons
  - morphology of all main DRG neuronal subtypes

NEXT STEP...

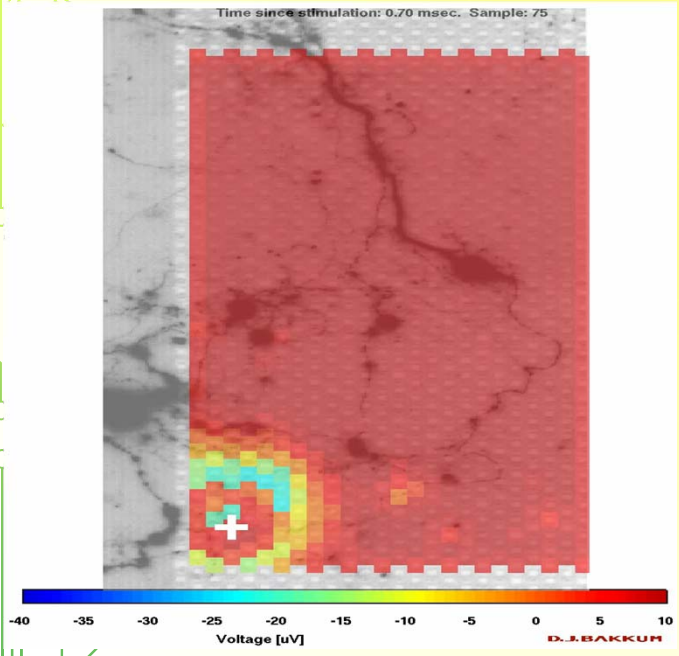
Micro-pillar substrates (MPS) ↔ **Micro-electrode arrays (MEA)**



For monitoring growth and morphology of DRG neurons



For high-resolution electrophysiological recordings



Micro-electrode arrays (MEAs)

based on integrated complementary metal oxide semiconductor (CMOS) allowing us to track axonal AP propagation with high spatio-temporal resolution

- ✓simultaneous stimulation and recording of any neuron lying on the array surface
- ✓non-invasive
- ✓for duration of months
- ✓accros hunderds of electrodes

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